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Marine hitchhikers: a preliminary study on invertebrates unintentionally transported via the international pet trade

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Abstract

The pet trade in aquatic organisms is a significant source of non-indigenous species introductions. In comparison with ornamental animals, unintentionally transported invertebrate assemblages are easily overlooked by traders and keepers. Moreover, hitchhiking species detection and identification is difficult even for experts. The densities of "hitchhikers" in aquaria may be relatively higher than those in the wild. These phenomena are known in freshwater aquaria but poorly studied in marine ones. We found 17 species of non-ornamental marine invertebrates in one of the leading importers of aquarium species in the Czech Republic in November 2017. The set comprised six gastropods, two bivalves, three cnidarians, two echinoderms, two crustaceans, and two polychaete worms. In one case, a symbiont was also detected, associated with the host "hitchhiker". No "live rocks" are traded by the surveyed wholesaler. Thus, the found animals were not imported together with this item as larvae or eggs. Contrary to the transport of targeted ornamental species, it is clear that transport of "hitchhikers" is occurring despite standard legislative regulations and should be brought to the attention of conservationists, wildlife managers, policymakers and other stakeholders.

Keywords

aquarium, biological invasion, invertebrate, non-ornamental species, symbiont

Introduction

While a majority of cultured and captured aquatic animals are exploited for human consumption, ornamental aquaculture is also an important and expanding sector of this industry (Padilla and Williams 2004). The keeping of aquatic animals and plants in aquaria is one of the most popular hobbies in the world (Tlusty 2002; Maceda-Veiga et al. 2016; Novák et al. 2020). In contrast with ornamental freshwater animals, marine fishes and invertebrates are mainly collected in the wild and millions of individuals of thousands of species are removed mainly from tropical coral reefs year by year (Rhyne et al. 2017). For this reason, researchers focussing on the exploitation of marine resources have proposed some suggestions on how to improve the sustainability of many harvested species, especially those not listed in the Convention on the International Trade in Endangered Species (CITES, www.cites.org), for example on the collecting of coral fish larvae in the wild for subsequent culture and stocking in aquaria as ornamentals (Bell et al. 2009; Lucas and Southgate 2019). The improvement of breeding in captivity is also highlighted but it is still only feasible for a few species due to a lack of proper methods and technologies (Tlusty 2002; Olivotto et al. 2011). Generally, decision-makers regulate the trade with marine biota including ornamental animals both locally and internationally, such as in the European Union (Duffy 2016) or the member countries in case of The International Council for the Exploration of the Sea (ICES) (Gollasch 2007).

However, some species may behave as invaders when they are released or they escape to a new locality beyond their native range. Even if there is still a debate on the pathway by which the species was introduced, the most highlighted invasive species in this regard, lionfishes (*Pterois volitans* and *P. miles*) invaded the Atlantic Ocean in the 1990s with devastating consequences for native benthic fauna (Albins and Hixon 2008, 2013; Green et al. 2012). Also, the aquarium origin of the green alga *Caulerpa taxifolia* introduced to the Mediterranean Sea can be mentioned (Jousson et al. 1998).

Aquatic organisms subjected to trade for ornamental purposes are transported intentionally (i.e., deliberately) and their invasion potential is known or could be evaluated. Unfortunately, the invasion potential of associated symbionts of intentionally transported species or faunal assemblages unintentionally transported with the targeted species (hereafter called "hitchhikers") are mostly overlooked. In comparison with the freshwater pet trade, where this phenomenon is well known (Rixon et al. 2005; Duggan 2010; Patoka et al. 2016a, b; Duggan et al. 2018), studies on unintentionally transported marine animals are lacking except for "live rocks" and locally transported organisms. The "live rocks" (marine rocks and old coral skeletons traded and used in marine aquaria for biological filtration as well as artificial reef substrate for other organisms and aesthetic functions) serve as a reservoir for a variety of marine microorganisms and invertebrates transported internationally as larvae or eggs (Padilla and Williams 2004; Calado and Narciso 2005; Walters et al. 2006). Various "hitchhikers" were found randomly transported with the locally traded popular marine aquarium green macroalga *Chaetomorpha* sp. in Florida (Odom 2012). The need for

further detailed study is essential because restrictions and regulation of unintentionally transported biota seem to be ineffective or simply impossible due to difficulties in species detection and identification. Interestingly the densities of "hitchhikers" in aquaria may be relatively high in comparison to the natural density of the same species in the wild (Ernst et al. 2011).

Many "hitchhiking" species have proven to be quite hardy. They are able to survive transport in sub-optimal conditions, as was documented by various aquatic animals found alive in boxes with water hyacinths (*Eichhornia crassipes*) shipped without water from Indonesia to the Czech Republic (Patoka et al. 2016b). Moreover, their invasion potential is in many cases high compared to ornamentals (Patoka et al. 2017). For the successful invasion of any aquarium species, individuals must overcome a series of sequential obstacles including transportation, release or escape from the tank to a new locality, and establishment of a new population in the wild. Although "hitchhikers" such as organisms from "live rocks" can generally improve the water quality in the tank (Yuen et al. 2009), some of them, typically species that pose a threat to fish and other aquarium inhabitants, are unwanted by hobbyists (Corsini-Foka et al. 2013). As a consequence, their release from the aquarium to a new locality is possible, as in the case of the toxic coral reef crab *Actaeodes tomentosus* in the Mediterranean Sea (Corsini-Foka and Kondylatos 2015).

Although there is no invasion risk of marine species in landlocked countries, animals can pass through the wholesaler-wholesaler or wholesaler-customer links and can thus be transported from an importer in the landlocked country to a coastal region where the invasion becomes a real threat as the secondary introduction. This is also true for "hitchhikers". It was previously noted that despite their small size, aquatic "hitchhikers" can significantly affect the ecosystems which they invade (Duggan 2010).

Improving knowledge about this overlooked part of the international pet industry can help to establish effective management strategies to reduce introduction rates. The Czech Republic is known as one of the leading importers for aquatic ornamental species and re-exporting many of these animals to other European countries (Kalous et al. 2015; Evers et al. 2019). Since we were alerted by the staff of the Czech wholesaler importing marine animals for ornamental purposes about an occurrence of "hitchhiking" creatures in their aquaria, we decided to survey the ornamental marine organisms there to determine which species are transported and stocked unintentionally as a preliminary study possibly resulting in future risk assessment of found taxa.

Methods

Data collecting

In 2017, we surveyed on two sampling occasions (on 6 and 20 November) 30 aquaria containing marine animals in the premises of the leading wholesale trader of ornamental organisms in Prague, in the Czech Republic, in business from 1990. First, we inter-

viewed staff (three persons) about "hitchhiking" creatures referred to as unintentionally imported. Subsequently, we visited the facility and these organisms were visually observed in tanks. Next, with the use of soft entomological tweezers, five individuals per species were sampled if possible. Finally, we did additional detailed searches to find more taxa in aquaria. The minimum size of organisms considered was 5 mm. These creatures were not transported with "live rocks" because these rocks are not traded in the surveyed wholesalers. Individuals were photographed and selected specimens were preserved in pure alcohol for later identification. The staff was asked about the origin of found organisms.

DNA analysis

For species identification, one individual of each collected taxon was separately DNA sequenced. DNA was isolated from ethanol-preserved tissue using DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer´s instructions. The mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using primers jgLCO1490 5'-TITCIACIAAYCAYAARGAYATTGG-3' and jgHCO2198 5'-TAIACYTCIGGRTGICCRAARAAYCA-3' (Geller et al. 2013). DNA extraction and amplification were processed according to (Patoka et al. 2016c). DNA was sequenced using the Macrogen sequencing service (www.macrogen.com). Chromatograms were assembled and checked for potential errors using BioEdit 5.0.9 software (Hall 1999). The obtained DNA sequences have been submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/). The Basic Local Alignment Search Tool (BLAST) was employed to find similarities in sequences in GenBank. The result was obtained in the form of a ranked list based on a normalized percent identity score, followed by individual sequence alignments (Madden 2013).

Results

In total, we found 17 "hitchhiking" taxa from six animal groups; six gastropods, two bivalves, three cnidarians, two echinoderms, two crustaceans, and two polychaete worms, in the leading wholesale trader of ornamental marine organisms in the Czech Republic (Fig. 1). Based on interviews with staff, we immediately found most of the "hitchhiking" species in the suggested tanks with no difficulty, but some species were not found in numbers of five or more individuals (details in the next paragraph). Just one more taxon was subsequently found through detailed inspection of the tanks and this was of a tiny size less than 5 mm (*Cymodoce* sp., Fig. 1O). From all samples sequenced for species identification (Table 1), in six individuals the PCR amplification was not successful (samples Nos. 212–214, 218, 221 and 222, hence not included in Table 1; these taxa were identified morphologically on the certain level such as bivalves etc.). For the remainder, the obtained COI fragments matched with the publicly avail-

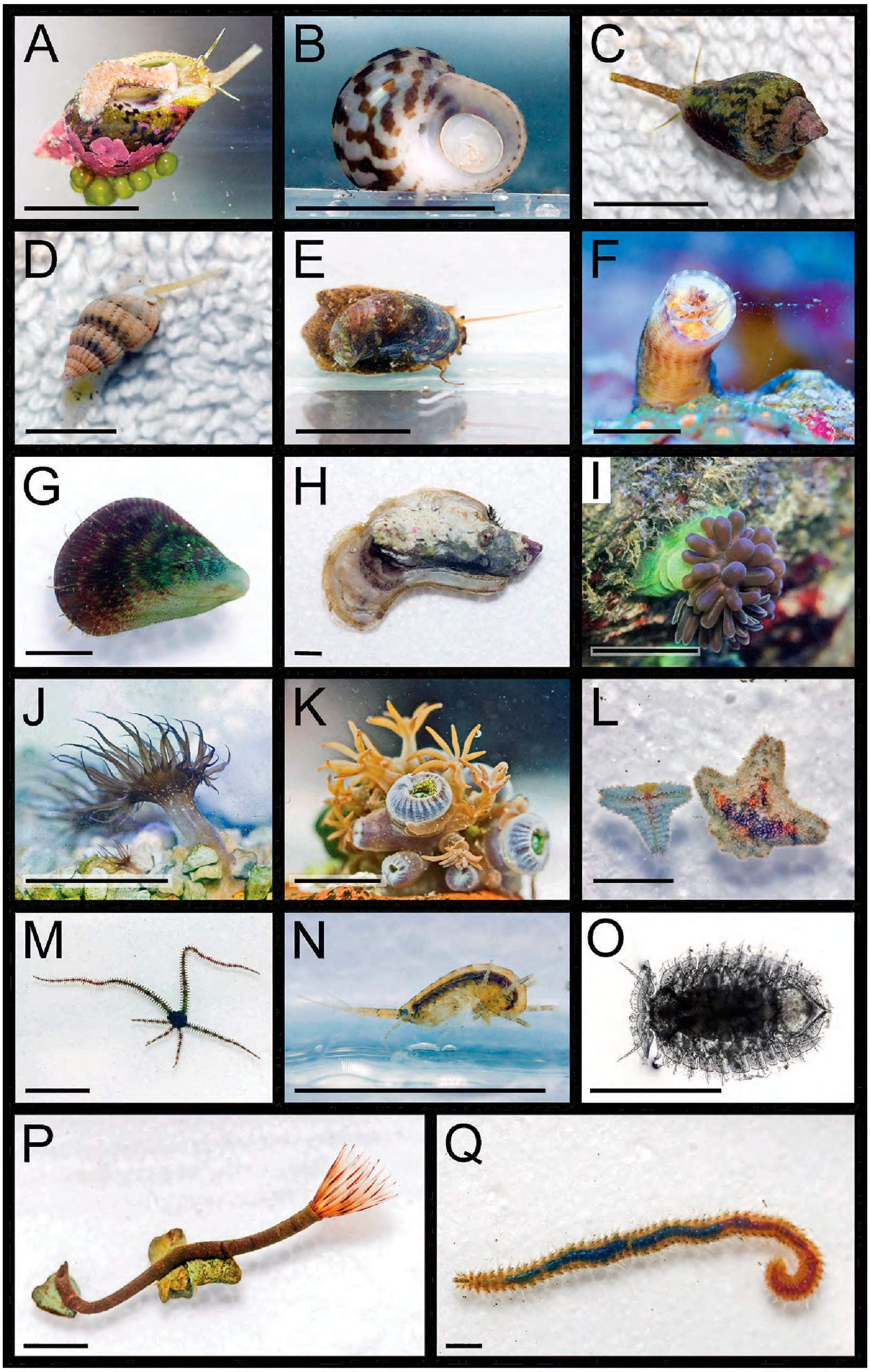

Figure 1. Found marine invertebrates **A** dove snail *Amphissa* / *Columbella* sp. with shell partly encrusted by algae **B** gastropod *Collonista* sp. **C** unidentified gastropod **D** unidentified gastropod **E** top-snail *Stomatella* sp. **F** worm snail *Serpulorbis* / *Thylacodes* sp. **G** unidentified bivalve **H** bivalve *Isognomon legumen* **I** unidentified sea anemone, possibly *Anemonia* cf. *manjano* **J** unidentified glass anemone, possibly *Aiptasia* sp. **K** soft coral *Acrossota amboinensis* **L** unidentified starfish, possibly *Asterina* sp. (left: typical three-armed regrown individual, right: individual with regenerated arms) **M** brittle star *Ophiocomella* sp. with four regenerated arms **N** amphipod *Niphargus* sp. **O** isopod *Cymodoce* sp. **P** fanworm *Bispira* sp. **Q** fireworm *Eurythoe* sp. Scale bars: 5 mm (**A–N**, **P–Q**), 1 mm (**O**).

Table 1. Identification of "hitchhikers" using DNA analysis; ID of the sample; GenBank: accession number; Taxon: name of the identified genus or species; BLAST: used Basic Local Alignment Search Tool and references.

ID	GenBank	Taxon	BLAST			Reference
			Query cover	Ident	Accession	
211	MT802127	*Columbella* sp. / *Amphissa* sp.	99% / 99%	87% 87%	KT753999.1 KF644285.1	Couto et al. (2016) / Layton et al. (2014)
215	MT802128	*Collonista* sp.	97%	92%	AM049345.1	Williams and Ozawa (2006)
216	MT802129	*Bispira* sp.	88%	83%	LT717721.1	Wood et al. (2017)
217	MT802130	*Stomatella* sp.	94%	98%	KX277585.1	Uribe et al. (2017)
219	MT802131	*Niphargus* sp.	98%	82%	KF719246.1	Esmaeili-Rineh et al. (2015)
220	MT802132	*Eurythoe* sp.	93%	99%	KY630466.1	Tilic et al. (2017)
223	MT802133	*Ophiocomella* sp.	100%	88%	KU895196.1	Hugall et al. (2015)
224	MT802134	*Thylacodes* sp. / *Serpulorbis* sp.	79%/90%	98% 85%	HM453709.1 AY296830.1	Faucci et al. (not published) / Colgan et al. (2003)
225	MT802137	*Isognomon legumen*	98%	100%	KX713469.1	Combosch et al. (2017)
226	MT802135	*Acrossota amboinensis*	95%	100%	GQ342379.1	Brockman and McFadden (2012)
227	MT802136	*Cymodoce* sp.	98%	80%	KJ410468.1	Khalaji-Pirbalouty and Raupach (2014)

able reference sequences (Table 1). The origin of sampled organisms was not clear but, based on information from wholesaler staff, the vast majority of them were imported "unseen" from Indonesia in several shipments with ornamental species. Subsequently, they were unintentionally released in aquaria where they grew and, in some cases, multiplied. Some found species had probably been living in the tanks for a long time.

In the surveyed tanks, we sampled five individuals of: *Columbella* sp. (Fig. 1A), *Collonista* sp. (Fig. 1B), two unidentified gastropod species (Fig. 1C, D), an unidentified starfish, possibly *Asterina* sp. (Fig. 1L), *Ophiocomella* sp. (Fig. 1M), *Niphargus* sp. (Fig. 1N), and *Cymodoce* sp. (Fig. 1O); two individuals of an unidentified sea anemone, possibly *Anemonia* cf. *manjano* (Fig. 1I); and one individual of: *Stomatella* sp. (Fig. 1E), a worm snail *Serpulorbis* / *Thylacodes* sp. (Fig. 1F), an unidentified bivalve (Fig. 1G), *Isognomon legumen* (Fig. 1H), an unidentified glass anemone, possibly *Aiptasia* sp. (Fig. 1J), *Acrossota amboinensis* (Fig. 1K), *Bispira* sp. (Fig. 1P), and *Eurythoe* sp. (Fig. 1Q).

Some of the unintentionally imported organisms were subsequently offered for sale (in the adult stage) by the wholesaler: a worm snail *Serpulorbis* / *Thylacodes* sp. (Fig. 1F), an unidentified sea anemone advertised as a majano anemone, probably *Anemonia* cf. *manjano* (Fig. 1I), a soft coral *Acrossota amboinensis* (Fig. 1K), and a fanworm *Bispira* sp. (Fig. 1P). The others were present in aquaria with ornamental species but not intended for trade. *Asterina* starfish were used as feed for the ornamental harlequin shrimp *Hymenocera picta* (Fig. 2). In many molluscs, the shells were partly encrusted with algae (Fig. 1A). Fireworms *Eurythoe* sp. (Fig. 1Q) were found in the substrate and also hidden in an empty snail shell.

In one case of the isopod *Cymodoce* sp. (Fig. 1O), a "hitchhiker of a hitchhiker" (probably ectocommensal) was detected since this isopod was associated with its host, the fanworm *Bispira* sp. (Fig. 1P). An assemblage of five *Cymodoce* individuals was collected on the surface of a single *Bispira* host.

Figure 2. The obligate and voracious echinoderm predator *Hymenocera picta* (harlequin shrimp) turning an *Asterina* starfish upside-down and eating the soft tissue from the central disc.

Discussion

We found several imported marine "hitchhikers" occurring in tanks with ornamental species stocked by a wholesaler in the Czech Republic. Since no "live rocks", "bio-rocks" or any other such substrates are imported and traded by the surveyed wholesaler, the animals found could not have been imported together with this item as eggs or larvae. Some of the found "hitchhikers" were subsequently traded or used as feed for other animals, while the vast majority were living in the tanks as non-utilized animals. It is estimated that millions of marine animals are captured in coral reefs and associated habitats each year for ornamental purposes (Rhyne et al. 2017), nevertheless, there are no estimations of the quantity of unintentionally removed and transported "hitchhikers". Our preliminary findings suggest that this pathway of non-ornamental marine species introduction is important but mostly overlooked and that a quantitative analysis should be conducted in the future.

Moreover, some species may be harmful to other organisms in the tank and, in some cases, also to the keeper. The large and iridescent fireworms such as the found *Eurythoe* sp. (Fig. 1Q) are covered by dense setae capable of penetrating human skin upon epidermal contact and are responsible for skin inflammation and painful "bristle-worm stings" (Halstead 1978). Fireworms are therefore unwanted inhabitants in reef aquaria (Tilic et al. 2017). Although some authors have suggested that these polychaetes are urticating (covered by bristles which can be ejected toward a potential attacker) rather than toxic (Penner 1970; Eckert 1985; Tilic et al. 2017), a complex mixture of toxins

used by fireworms for their defence against predators was recently found (Verdes et al. 2017). Since fireworms were found hidden in an empty snail shell in the aquarium, their occasional unintentional translocation cannot be excluded.

"Hitchhiking" glass anemones from the genus *Aiptasia* (Fig. 1J) are small anemones some 3 cm in diameter. These highly resistant and aggressive cnidarians, described by some keepers as the worst "nightmare" one can have in the aquarium (McBirney 2013), are unpopular in reef aquaria because of their ability to dominate in the tank. In nutrient-rich tanks with good lighting, *Aiptasia* anemones quickly reproduce asexually by pedal laceration, and powerfully sting invertebrates and unwary fish to push them away, causing their mortality in many cases (Rhyne et al. 2004). The found majano anemones (*Anemonia* cf. *manjano*, Fig. 1I) are attractively coloured even as pest anemones in reef aquaria. They are less prolific than *Aiptasia* anemones and they are therefore generally perceived as ornamentals by hobbyists. However, similarly to *Aiptasia*, they may be very harmful to other sessile invertebrate inhabitants in the aquarium through their powerful stings (Ram 2013).

Ophiocomella brittle stars (Fig. 1M) and *Asterina* starfishes (Fig. 1L) can reproduce asexually by fragmentation (i.e., fissiparous reproduction); their bodies split apart losing one or two arms at a time, which regrow as new individuals of asymmetrical appearance (Mladenov et al. 1983; Wilkie et al. 1984; Achituv and Sher 1991). Therefore, both could be transported as overlooked fragments. Various *Asterina* species prey on corals but also grazing on algae covering the shells of "hitchhiking" dove snails *Amphissa* / *Columbella* sp. (Fig. 1A) was observed by wholesaler staff.

Very popular as an ornamental species in reef aquaria, mainly due to its attractive colouration, is the harlequin shrimp *Hymenocera picta* (Fig. 2). This shrimp is an obligate and voracious echinoderm predator (Wickler 1973). To feed it, hobbyists must have plenty of starfishes available (Prakash and Kumar 2013). For instance, the estimated annual costs of starfish for feeding one pair of harlequin shrimps in the USA is $260–390 USD per year. Therefore, the pest *Asterina* starfish is popular as low-cost food for these shrimps, and "hitchhiking" starfish can be spread in this way to other tanks. This was also the case with the surveyed wholesaler in the Czech Republic who advertised *Asterina* starfish as a suitable feed for traded harlequin shrimps.

The isopod crustacean *Cymodoce* sp. (Fig. 1O) found associated with the polychaete fanworm *Bispira* sp. (Fig. 1P) can easily be overlooked and transported with its host, which can be traded as ornamental despite its first importation as a "hitchhiker". Although we have no details about the ecological relationship of *Cymodoce* with the fanworm, this isopod did not occur elsewhere in the aquarium. It was previously suggested that isopods from the same family (Sphaeromatidae) may live in polychaete tubes (Müller 1990). Therefore, the possibility that the collected *Cymodoce* sp. is an obligate symbiont primarily introduced to aquaria with its host fanworm should be examined in future studies.

We have mentioned above some possible pathways by which marine "hitchhikers" can spread via the pet trade. There are some effective methods to mitigate or eradicate their occurrence in aquaria, such as stocking commonly traded shrimps of the genus

Lysmata in reef aquaria as effective predators of "hitchhiking" glass anemones (Rhyne et al. 2004; Calado and Narciso 2005). Nevertheless, some hobby keepers might decide to release unwanted pests into neighbouring seas. Ornamental aquatic animals are re-exported from the Czech Republic to other European countries (Ploeg 2007; Kalous et al. 2015; Patoka et al. 2015) including coastal regions where invasions of marine biota may take place. Some "hitchhiker" populations have expanded rapidly and become dominant species in coral reef tanks, such as the found gastropods of the genus *Collonista* (Fig. 1B); these molluscs are perceived as a menace by owners of marine aquaria (see http://www.reefcentral.com/forums/showthread.php?t=2284901) and could be released.

There is then the potential for released "hitchhiking" species to behave as invaders when introduced to a suitable new locality in the wild. Certain species collected in this study or closely related to these species can be seen as examples of successful and fast multiplying creatures even if their introduction pathway was not via ornamental aquaculture. For instance, the starfish *Aquilonastra burtoni* (family Asterinidae) invaded the Mediterranean Sea and consequently caused the decline of a native congener *Asterina gibbosa* (Achituv and Sher 1991; Galil 2007). Conversely, the fanworm *Sabella spallanzanii*, native to the Mediterranean, has invaded the ocean around southern Australia and northern New Zealand. It is currently abundant in these areas and both ecological and economic impacts are expected (Wood et al. 2017) because, in high densities, it has the potential to compete with cultured gastropods (Currie et al. 2000; Murray and Keable 2013). A third example is the non-native vermetid worm-snail, *Thylacodes vandyensis*, which was recorded attached to the wreck of the USNS *Vandenberg* sunk as an artificial reef close to the coast of Key West, USA, to reduce pressure on the surrounding natural reefs. As vermetid snails influence the growth of corals (Shima et al. 2010; Tootell and Steele 2014) and serve as intermediate hosts for turtle blood flukes (Cribb et al. 2017), they are of concern to wildlife managers (Bieler et al. 2017). On the other hand, the limited habitat match between source region (usually, tropical reefs) of traded or "hitchhiking" species and possible introduction regions reduces the probability of establishment in the wild in the temperate zone. Hence, tropical regions are most at risk from such species.

The replacement of potentially invasive species by low-risk species in aquaria is traditionally mentioned as a possible and safe way to mitigate the risk of biological invasions of ornamental organisms. Nevertheless, this approach is not feasible with "hitchhikers" which are mostly undetected due to their tiny size, and their release with wastewater is likely (Odom and Walters 2014). It must be noted that the problem is probably underestimated because, as well as macroinvertebrates, large quantities of microorganisms associated with their hosts are also likely to be transported unseen via the ornamental trade (Barille et al. 2017). In line with a previous publication on the effectiveness of legislative restrictions for aquatic pets (Patoka et al. 2018), the transportation of "hitchhikers", unlike ornamental species, is mostly uncontrollable by standard regulations. Since the majority of ornamental marine animals are imported into the United States, Europe, and Japan, their further monitoring and analyses of related risks at least in these countries are strongly recommended.

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